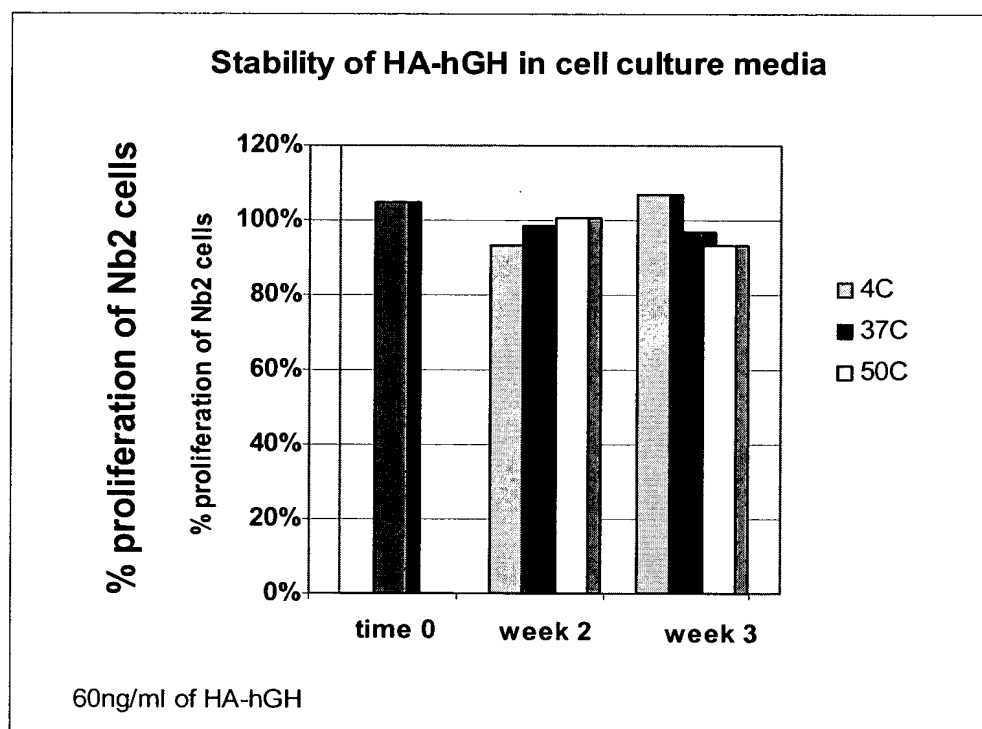


**Figure 1**

**Figure 2**

3/18

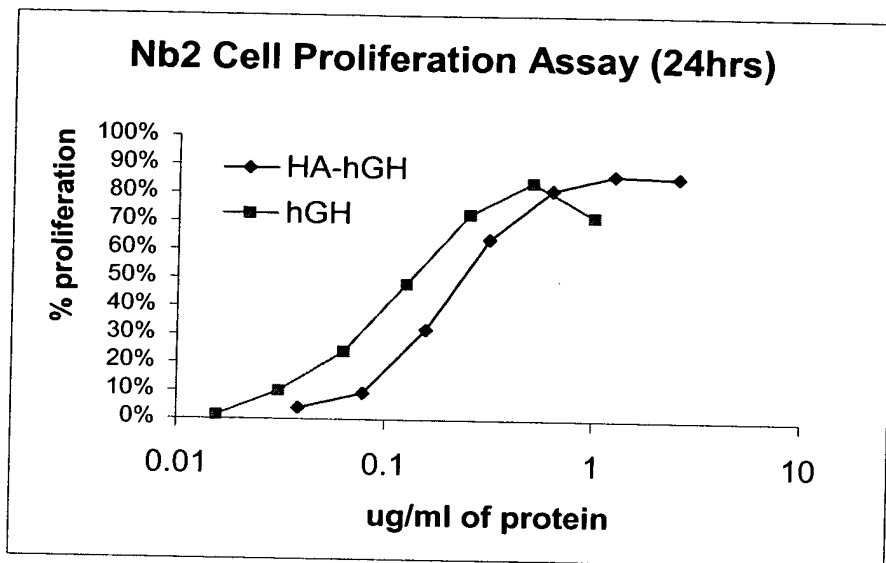


Figure 3A

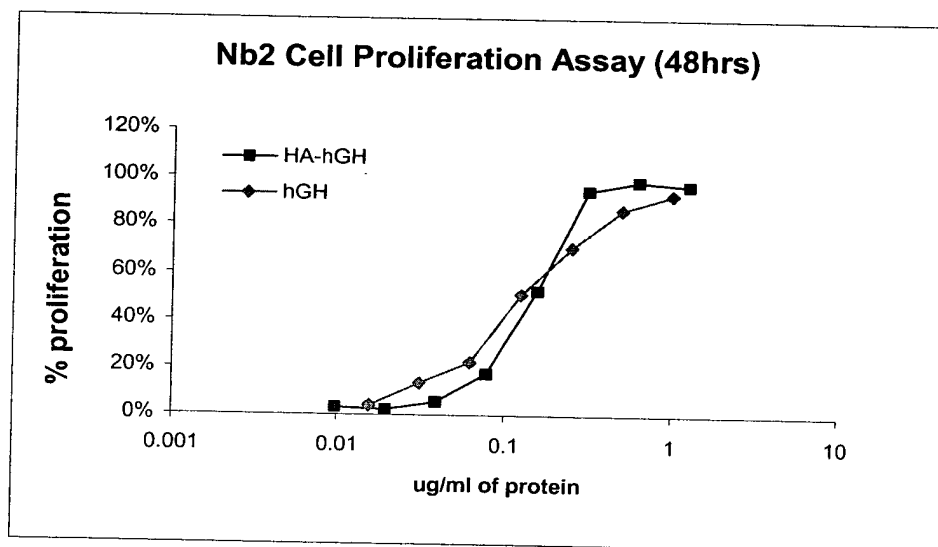


Figure 3B

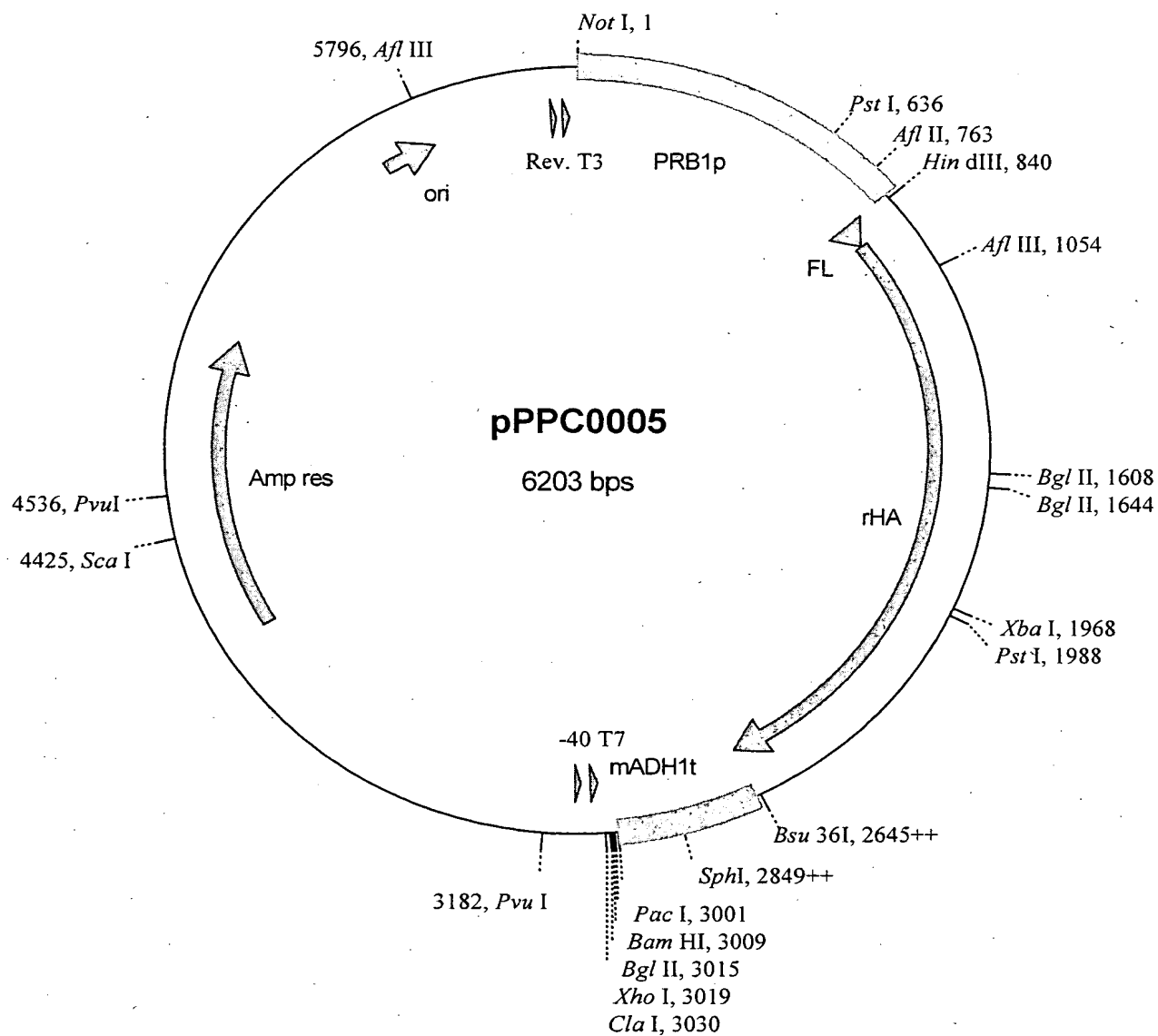


Figure 4

5/18

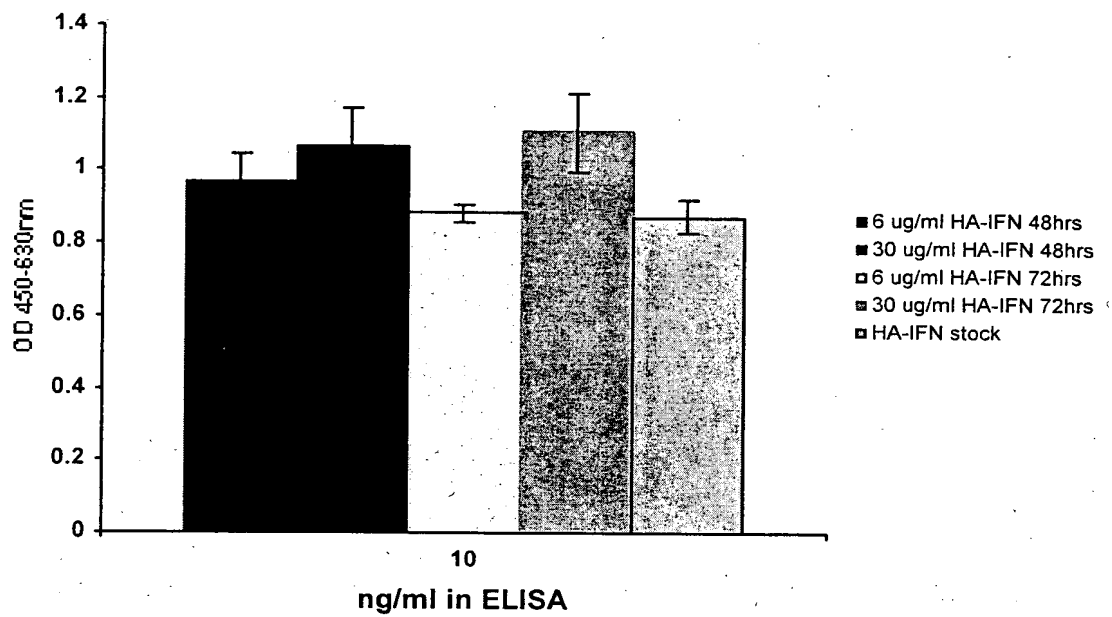


Figure 5

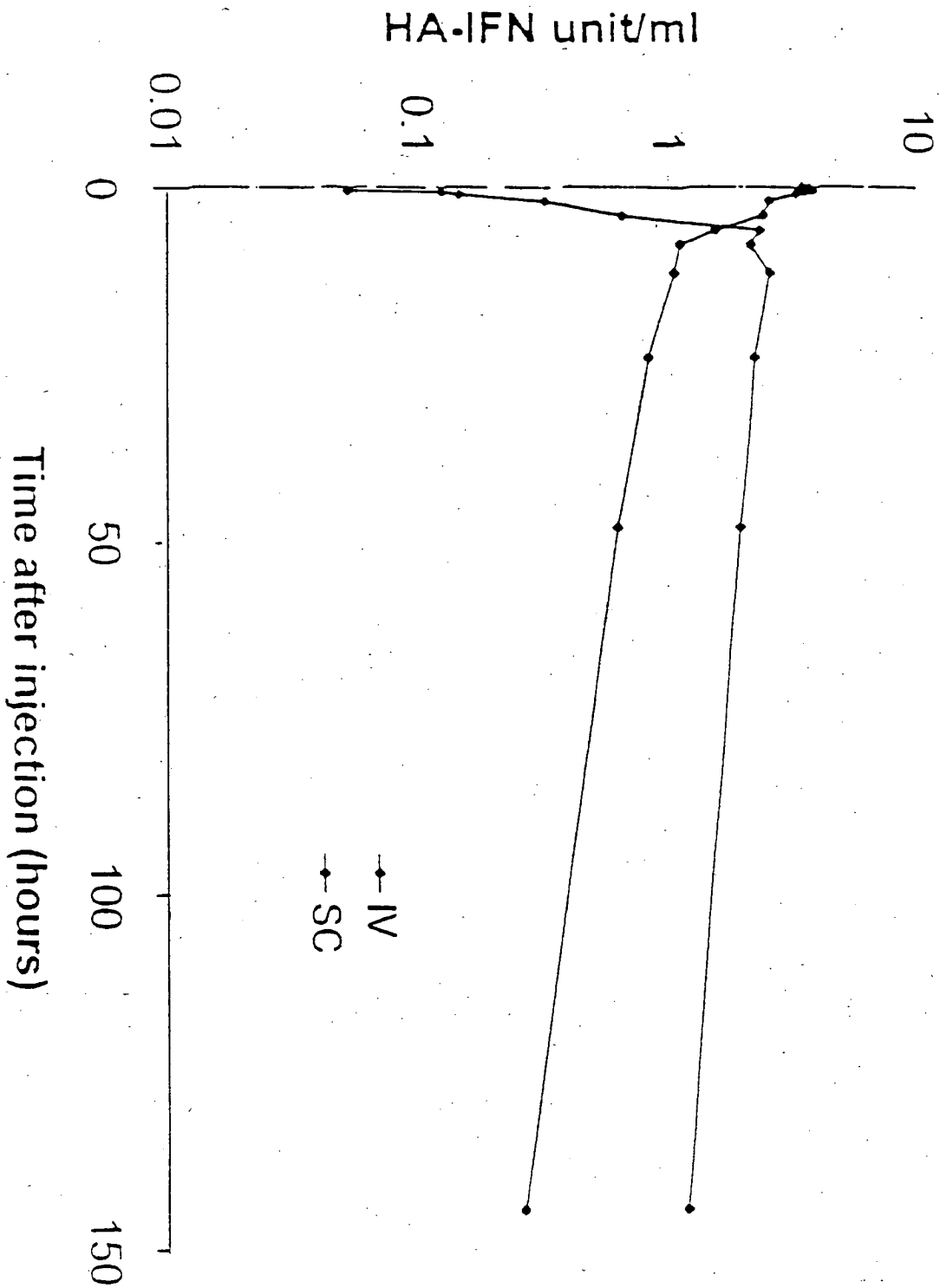
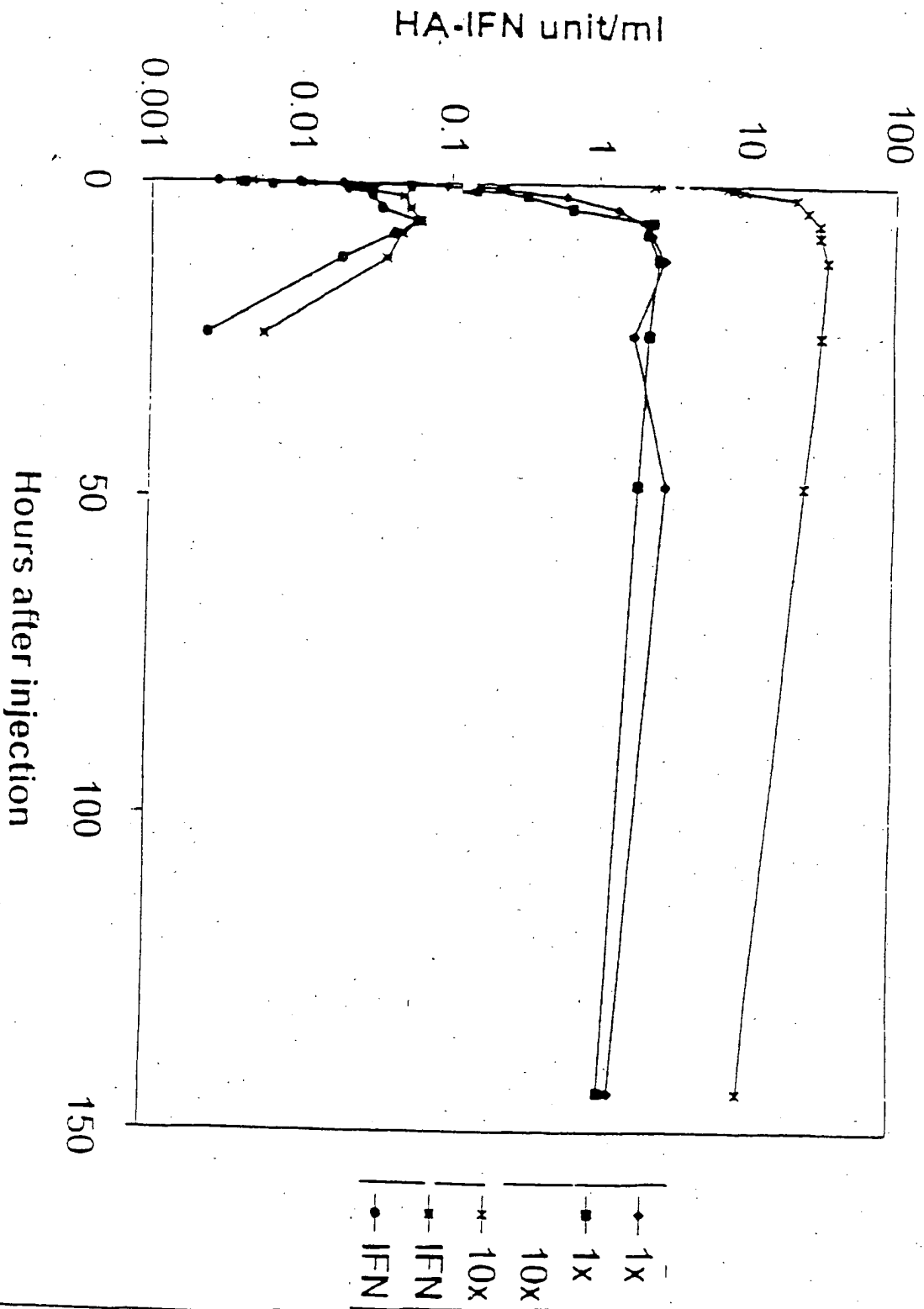


Figure 6

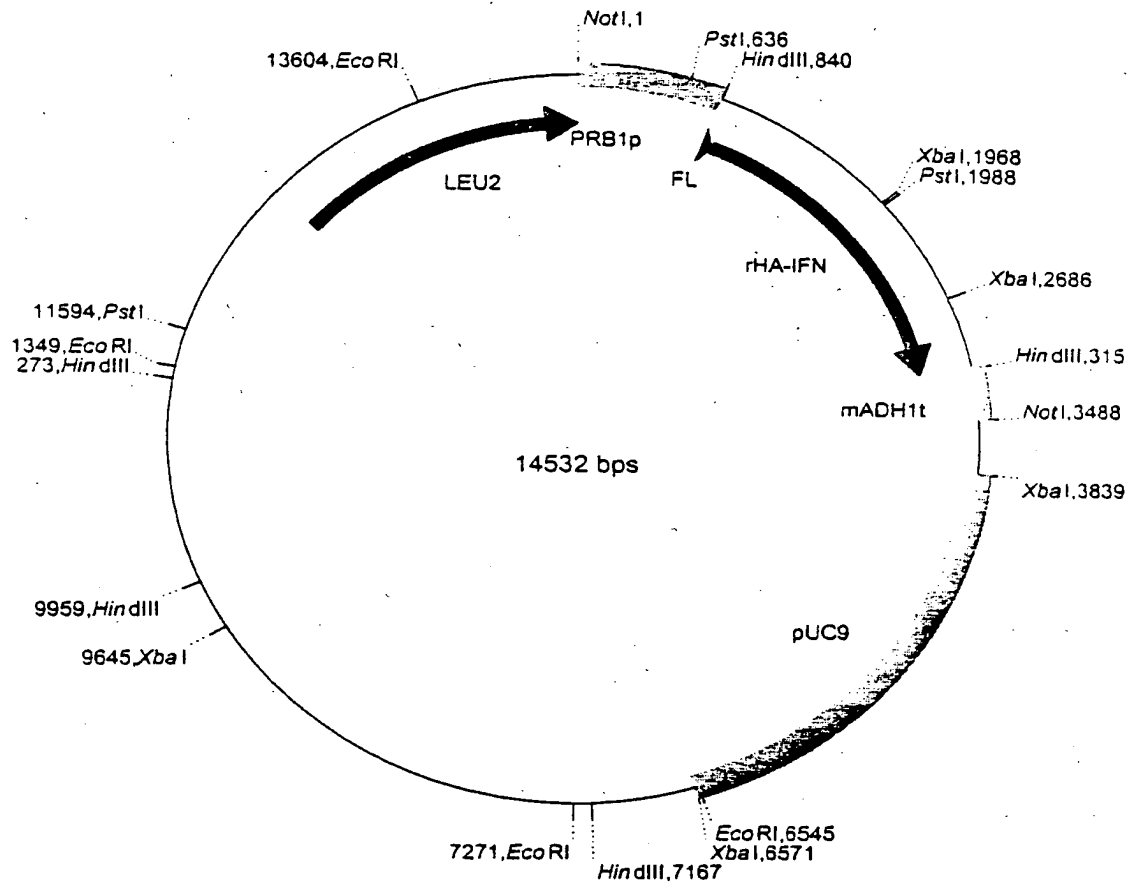
Figure 7



HA-IFN unit/ml

Hours after injection

1x  
1x  
10x  
10x  
IFN  
IFN



**Figure 8.** The HA-IFN $\alpha$  expression cassette in pSAC35. The expression cassette comprises

*PRB1* promoter, from *S. cerevisiae*.

Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MF $\alpha$ -1 leader.

HA-IFN $\alpha$  coding sequence with a double stop codon (TAATAA)

*ADH1* terminator, from *S. cerevisiae*. Modified to remove all the coding sequence normally present in the *Hind III*/*Bam*HI fragment generally used.

**Figure 8**



9/18

**Localisation of 'Loops' based on the HA Crystal Structure  
which could be used for Mutation/Insertion**

```

1    DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
      HHHHH HHH      HHH HHHHHHHHHHH      HHHHH HHHHHHHHHHH

      I                      II                      III
51   KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
      HHHHH      HHHHH HHHHH      HHHH H      HHHH

101  CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
      HHHH      H      HHHHHHHHH      HHHHHHHHH HHHHH

      IV
151  APELLFFAKR YKAAFTECCO AADKAACLLP KLDEL RDEGK ASSAKQRLKC
      HHHHHHHHHHH HHHHHHHHH      HHHHH HHHEHHHHHH HHHHHHHHHHH

      V
201  ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
      HHHHH      HH HHHHHHHHHHH HH      HHH HHHHHHHHHHH HHHHHH      HH

      VI                      VII
251  LECADDRADL AKYICENODS ISSKLKECCE KPLEKSHCI AEVENDEMPA
      HHHHHHHHHHH HHHHH      HHHHH      HHHHHHH H

301  DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLRLA
      HHHH      HHHHHH      HHHHHHH HHHHHH      HHHHHHHH

      VIII
351  KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
      HHHHHHHHHHH      HH      H      HHHHH HHHHHHHHHHH HHHHHHH

      IX
401  YKFQNALLRV YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
      HHHHHHHHHHH HHHH      H HHHHHHHHHHH      HHH      HHHHHHHHH

      X                      XI
451  DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
      HHHHHHHHHHH HHHHH      HHHHHHHHHH      HHHHHHHH

501  EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
      HHH      HHH HHHHMMEHHH HHH      HHHHHHHH

      XII
551  FAAFVEKCCCK ADDKETCFAE EGKKLVAAASQ AALGL
      HHHHHHHHH      HHHH HHHHHHHHHHH HH
  
```

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

**Figure 9**

Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151    APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC  
       HHHHHHHHHH HHHHHHHHHH            HHHHH HHHHHHHHHHH HHHHHHHHHHH

IV

151    APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDEL RDEGK ASSAKQRLKC  
       HHHHHHHHHH HHHHHHHHHH            HHHHH HHHHHHHHHHH HHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

**b. Insertion (or replacement) of Randomised sequence into Loop IV.**

(X)<sub>n</sub>



IV

151    APELLFFAKR YKAAFTECCQ AADKAACLLP KLDEL RDEGK ASSAKQRLKC  
       HHHHHHHHHH HHHHHHHHHH            HHHHH HHHHHHHHHHH HHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

**Figure 10**

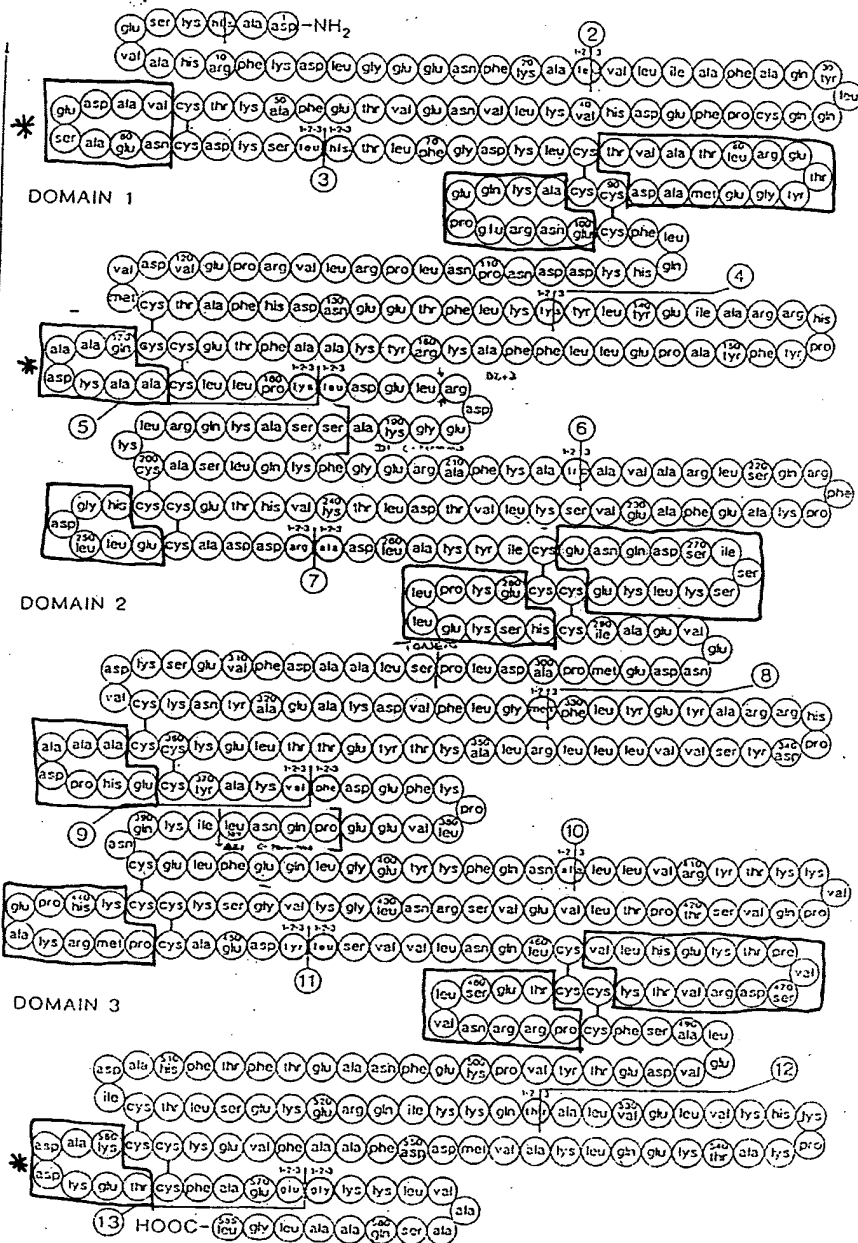
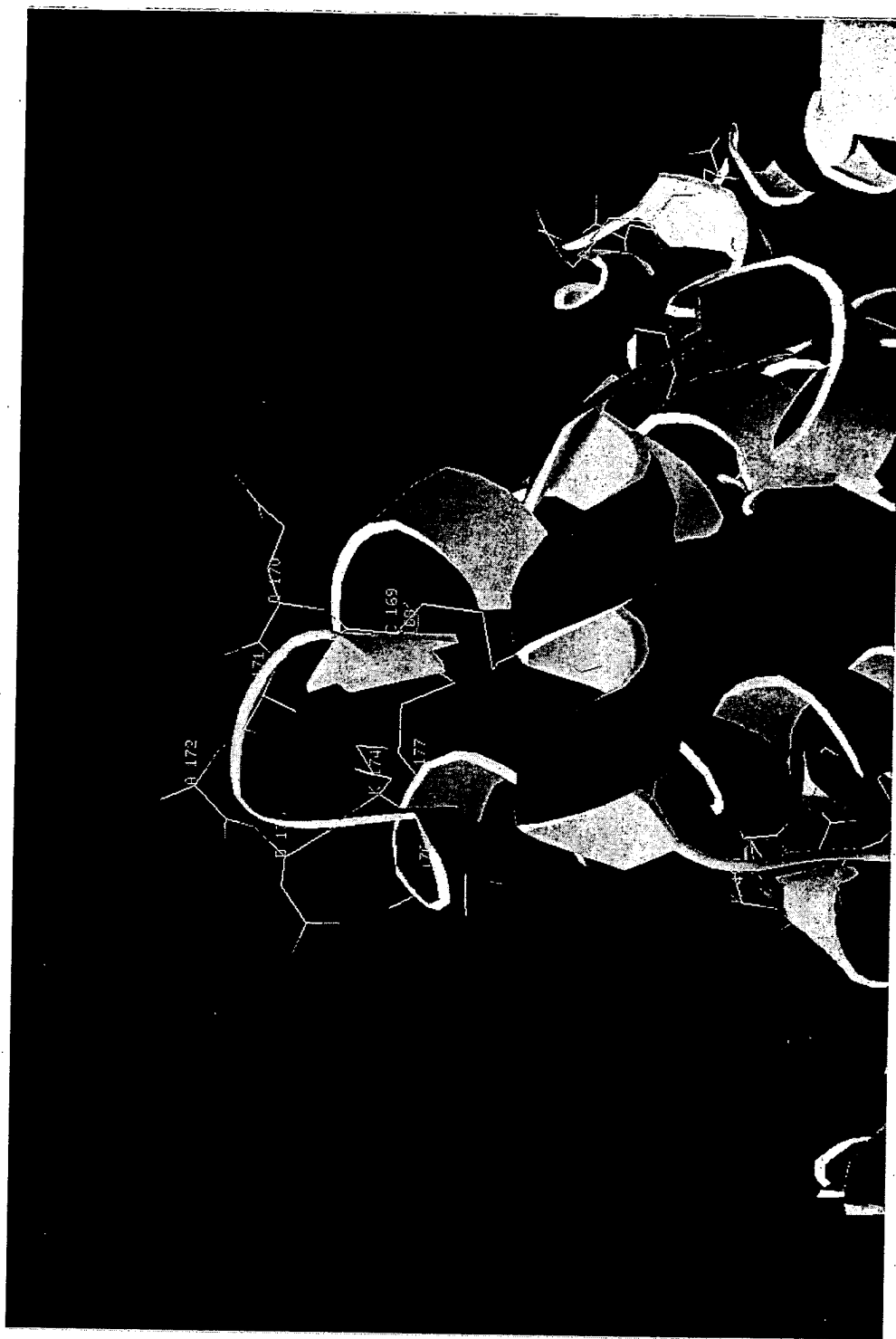
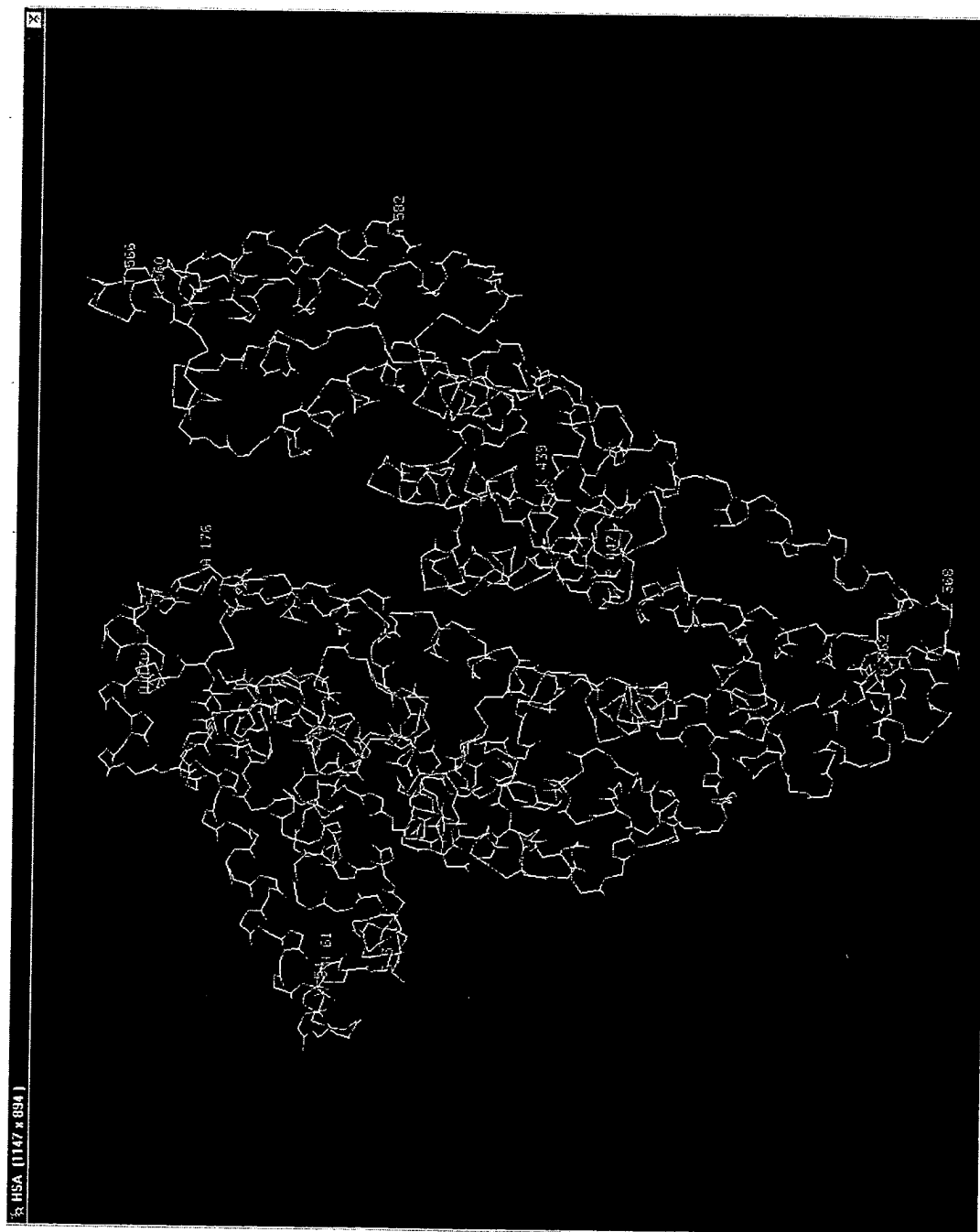


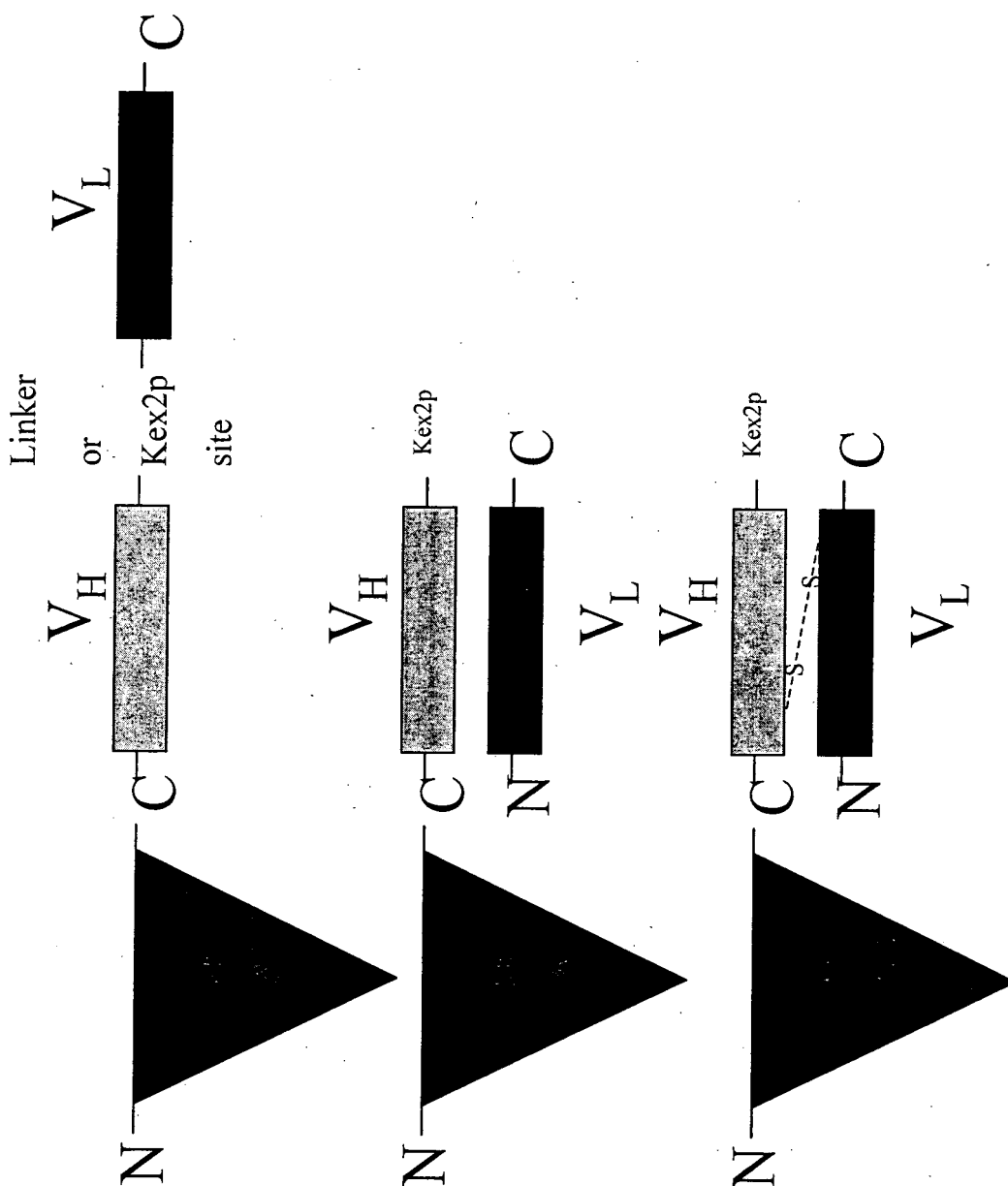
Figure 11



Disulfide bonds shown in yellow

**Figure 12: Loop IV Gln170-Ala176**





**Figure 14: Schematic Diagram of Possible ScFv Fusions**  
 (Example is of a C-terminal fusion to HA)

1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60  
1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120  
21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180  
41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240  
61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300  
81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360  
101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420  
121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480  
141 E I A R R H P Y F Y A P E L L L F F A K R 160

Figure 15A

16/18

481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540  
 161 Y K A A F T E C C Q A A D K A A C L L P 180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600  
 181 K L D E L R D E G K A S S A K Q R L K C 200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660  
 201 A S L Q K F G E R A F K A W A V A R L S 220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720  
 221 Q R F P K A E F A E V S K L V T D L T K 240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780  
 241 V H T E C C H G D L L E C A D D R A D L 260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840  
 261 A K Y I C E N Q D S I S S K L K E C E 280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900  
 281 K P L L E K S H C I A E V E N D E M P A 300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960  
 301 D L P S L A A D F V E S K D V C K N Y A 320

Figure 15B



17/18

961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020  
 321 E A K D V F L G M F L Y E Y A R R H P D 340

1021 TAC TCT GTC CTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080  
 341 Y S V V L L L R L A K T Y E T L E K C 360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140  
 361 C A A A D P H E C Y A K V F D E F K P L 380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200  
 381 V E E P Q N L I K Q N C E L F E Q L G E 400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260  
 401 Y K F Q N A L L V R Y T K K V P Q V S T 420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320  
 421 P T L V E V S R N L G K V G S K C C K H 440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380  
 441 P E A K R M P C A E D Y L S V V L N Q L 460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440  
 461 C V L H E K T P V S D R V T K C C T E S 480

Figure 15C

18/18

1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500  
 481 L V N R R P C F S A L E V D E T Y V P K 500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560  
 501 E F N A E T F T F H A D I C T L S E K E 520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620  
 521 R Q I K K Q T A L V E L V K H K P K A T 540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT TTT GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680  
 541 K E Q L K A V M D D F A A F V E K C C K 560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740  
 561 A D D K E T C F A E E G K K L V A A S Q 580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782  
 581 A A L G L \* 585

Figure 15D